

# Aetiology of genital ulceration in the Gambia

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**SUMMARY** The aetiology of genital ulceration was studied in 104 unselected patients (94 men, 10 women) attending a sexually transmitted disease (STD) clinic in the Gambia. Chancroid was diagnosed in 54 (52%), syphilis in 23 (22%), lymphogranuloma venereum (LGV) in seven (7%), and herpes in six (6%). In 28 (27%) patients no diagnosis was reached. Ten (10%) patients were found to have both chancroid and syphilis, three (3%) had both LGV and syphilis, and one (1%) had both herpes and syphilis. Trimethoprim 160 mg and sulphamethoxazole 800 mg twice daily for one week was found to be effective in the treatment of chancroid. This regimen with a single intramuscular injection of benzathine penicillin 2.4 MIU is recommended for the treatment of genital ulcers in Gambian health centres without laboratory facilities.

The incidence of genital ulceration is extremely high in many parts of Africa, and in some areas is believed to be similar to that of urethritis.<sup>1-6</sup> Whereas most genital ulcers in developed countries are due to herpes simplex virus,<sup>7,8</sup> this has not been found to be the case in the African countries in which studies have been carried out.<sup>5,6,9</sup>

It has long been felt on clinical grounds that, even in areas of Africa where syphilis is highly prevalent, most genital ulcers are due to chancroid.<sup>1,4</sup> The clinical diagnosis of genital ulcers is unreliable, however,<sup>10,11</sup> and only in recent years have improved culture techniques enabled this impression to be confirmed microbiologically. *Haemophilus ducreyi* was isolated in Nairobi from 62% of 97 patients with genital ulcers,<sup>5</sup> and in Johannesburg from 61% of 102 patients.<sup>6</sup>

To our knowledge, the aetiology of genital ulceration has not been systematically studied in west Africa, although the isolation of *H. ducreyi* has been reported from three patients in Nigeria.<sup>12</sup> The aim of the present study was to investigate the aetiology of genital ulceration in patients attending a clinic for sexually transmitted diseases (STDs) in the Gambia.

## Patients and methods

### PATIENTS

The Gambia is a small predominantly Muslim country on the west coast of Africa between latitudes 13 and 14° N. At Fajara, on the coast, an STD clinic was established in the Medical Research Council hospital in 1980. The present study describes the findings in 104 unselected patients (94 men, 10 women) attending this clinic with genital ulceration. All were investigated by dark field microscopy, culture for herpes simplex virus and *H. ducreyi*, and serological tests for infection with *Treponema pallidum*, herpes simplex virus, or *Chlamydia trachomatis* serotypes L1-3.

### LABORATORY METHODS

#### Dark field microscopy

After the base of the ulcer had been cleaned with normal saline, a drop of exudate was placed on a slide and examined immediately under dark field illumination at a magnification of  $\times 400$ . *T. pallidum* was identified by its characteristic morphology and mobility.

#### Culture of herpes simplex virus

Swabs taken from the base of the ulcer were immediately expressed into viral transport medium containing 10% fetal calf serum and stored at  $-70^{\circ}\text{C}$ . They were subsequently inoculated on to Vero cells in minimum essential medium with 5% fetal calf serum, and the cells were examined daily for cytopathic effect for

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Accepted for publication 6 January 1987.

two weeks. Herpes simplex virus was identified by its characteristic cytopathic effect.

#### Culture of *H ducreyi*

The ulcer was cleaned thoroughly with sterile normal saline, and swabs were taken from the base of the ulcer. These were inoculated directly on to each of two culture media. One contained 10% sheep blood in Columbia agar base (Oxoid Ltd) and the other 7% chocolate horse blood in Columbia agar base. Each was enriched with 5% fetal calf serum and vitox (Oxoid Ltd) and made selective with vancomycin (3 mg/l).

Plates were incubated for up to 10 days at 33°C in a candle extinction jar containing a moistened paper towel. They were inspected daily and *H ducreyi* was identified by colonial morphology, Gram stain, and standard biochemical techniques.<sup>13</sup>

#### Cultures of *C trachomatis*

Swabs taken from the base of the ulcer were expressed immediately into sucrose phosphate (2SP) transport medium containing 10% fetal calf serum, and stored at -70°C. They were subsequently inoculated, with centrifugation, on to cycloheximide treated McCoy cells, which were stained with Giemsa and examined for inclusions after incubation for 48 hours.<sup>14</sup>

#### Serological tests

The Venereal Disease Research Laboratory (VDRL) test was performed according to standard techniques using undiluted serum and Wellcome reagent VD 02-03. The *T pallidum* haemagglutination assay (TPHA) was performed according to standard techniques using serum at dilutions of 1:80 and 1:160 with reagent obtained from Fujizoki Pharmaceutical Company, Tokyo.

Sera were screened for IgG antibodies to *C trachomatis* serotypes L1-3 at a dilution of 1:250. A microimmunofluorescence (MIF) test was used with egg grown chlamydial antigens kindly supplied by Dr M Tam.<sup>15</sup>

IgG antibodies to herpes simplex were measured by MIF in 28 patients from whom paired sera were available. Herpes simplex infected Vero cells were used as antigen, and sera were tested at doubling dilutions from 1:8 to 1:256.<sup>16</sup>

#### TREATMENT

All patients were treated with co-trimoxazole two tablets daily for one week (each tablet containing 80 mg trimethoprim and 400 mg sulphamethoxazole). They were also advised to wash their ulcers four times daily in salt water. Those who were dark ground or VDRL positive were also treated with either a single intramuscular injection of benzathine penicillin 2.4

MIU or 10 daily intramuscular injections of procaine penicillin 600 000 IU. They were asked to return for follow up one and four weeks after their first visit. Those who did not show a considerable improvement after one week were in most cases treated with the same regimen of co-trimoxazole for a further week.

#### Results

Of the 104 patients with genital ulceration seen, 10 were women (mean age 25.6, range 15 to 45) and 94 were men (mean age 28.9, range 17 to 75). None of the women had had sexual contact other than with a single regular partner. Of the men 42 (44%) appeared to have been infected by a prostitute or other casual sexual contact. All but two of the men were circumcised. Fifty two patients had received treatment before they were seen. In 10 patients this consisted only of topical application of antiseptic or antibiotic ointment, the other 42 had taken systemic antibiotics. In most cases treatment comprised less than 10 capsules of tetracycline or fewer than three injections of penicillin. Only six patients had received a course of treatment possibly effective against syphilis.

Table 1 shows the duration of symptoms when the patients were first seen. In 57 (55%) patients more than one ulcer was present. In 42 (40%) inguinal glands greater than 1 cm in diameter were noted, unilaterally in 29 and bilaterally in 13. In 12 cases these were fluctuant or discharging.

*H ducreyi* was isolated from 54 (52%) patients; 34 were isolation negative and in 16 cases cultures were overgrown with contaminants. Thus, excluding patients whose cultures were contaminated, *H ducreyi* was isolated from 54 of 88 (61%) patients.

Positive VDRL and TPHA test results suggestive of active syphilis were found in 22 (21%) patients; four of these were dark ground positive. A further patient, whose ulcer had been present for one week, was dark ground positive but serologically negative. Four patients had positive TPHA but negative VDRL results, which suggested a previous treponemal infection.

Seven patients had chlamydial antibody titres of 1:250 or greater, which suggested that they were

Table 1 Duration of genital ulceration reported at initial presentation of 104 patients

Duration	No of patients
< 1 week	22
1 week -	28
2 weeks -	16
3 weeks -	12
1 month -	14
2 months -	4
3 months +	8

suffering from lymphogranuloma venereum (LGV). Three of them also had serological evidence of syphilis.

Herpes simplex virus was isolated from four (4%) patients. In a further two patients there was a four-fold increase in antibody titre to herpes simplex virus; one of these patients also had syphilis. Table 2 summarises the diagnoses reached.

At least one follow up visit was made by 74 (71%) patients. Complete healing of the ulcers was noted at one week in 29 of these (39%) and at subsequent follow up in a further 20 (27%). Twenty one (28%) patients were noted to be improving after one week but were lost to subsequent follow up. Four, two of whom had positive serological test results for syphilis, showed no improvement at one week and were lost to follow up.

## Discussion

The incidence of genital ulceration in the Gambia is not known, but in our clinic we see comparable numbers of cases of genital ulceration and urethritis. Government health centres and hospitals in the Gambia treat 16 000 men with urethral discharge a year (Jack A, personal communication). As there are thought to be fewer than 200 000 adult men in the Gambia (1973 census), the incidence of urethritis is likely to be about 10% a year.

Syphilis is known to be prevalent in the Gambia, where endemic treponematoses are now rare. At an urban antenatal clinic a prevalence of 1% was found,<sup>17</sup> and at a rural clinic a prevalence of 11% (Mabey D, unpublished observation), diagnosed by positive VDRL and TPHA results. Nevertheless, we have found that only 22% of genital ulcers seen in our clinic are due to syphilis. The low proportion of dark ground positive patients is presumably due to the high incidence of previous medication.

The results of the present study are remarkably similar to those from Nairobi<sup>5</sup> and Johannesburg.<sup>6</sup> Excluding patients whose cultures were contaminated, we isolated *H ducreyi* from 61% of genital ulcers, compared with 62% in Nairobi and 61% in Johannesburg, and herpes simplex virus from 4%

compared with 4% in Nairobi and 9% in Johannesburg. The corresponding figures for syphilis are 22% (the Gambia), 12% (Nairobi), and 15% (Johannesburg).

We have found that LGV is not a common cause of genital ulceration in the Gambia, which agrees with the figure of 1% in Johannesburg. Classically, it is said that extremely high titres of circulating anti-chlamydial antibody are found in this condition;<sup>18</sup> it has been shown in Swaziland, however, that *C trachomatis* may be isolated from genital ulcers of patients with low titres of circulating antibody detected by MIF.<sup>19</sup> Whether these are true cases of LGV or represent urethral contamination of the ulcers is not known. We have isolated an LGV strain of *C trachomatis* (L2) from a patient with ophthalmia neonatorum in the Gambia (Forsey T, Mabey D, unpublished observation), but failed to isolate chlamydiae from 46 genital ulcers from which uncontaminated specimens were obtained (Mabey D, unpublished observation). Of seven patients in the present study with chlamydial antibody titres of more than 1/250, three also had serological evidence of syphilis. Of the other four patients, only one had a clinical diagnosis of LGV. On the other hand, we have isolated *H ducreyi* from the buboes of three patients with a clinical diagnosis of LGV who did not have ulcers at presentation. We conclude, as have others,<sup>11</sup> that the clinical diagnosis of genital ulcers in the tropics is unreliable.

It is not clear why herpes simplex virus should be isolated from such a small proportion of ulcers in Africa, when it is the commonest pathogen isolated in developed countries.<sup>7,8</sup> The low isolation rate may be due to the relatively late presentation of genital ulcers in African clinics (table 1), but information on length of history has not been given in most studies. Alternatively, the high prevalence of serum antibody to herpes simplex virus types 1 and 2 in African communities may indicate a degree of immunity. It has been shown that neutralising antibodies to herpes simplex virus type 2 are often acquired in childhood in one African city.<sup>20</sup>

Méheus has proposed guidelines for the treatment of genital ulcers in African clinics without laboratory facilities.<sup>21</sup> We have confirmed his view that co-trimoxazole two tablets twice daily for one week, combined with saline washes, is an effective regimen for the treatment of chancroid; this is reflected in our in vitro findings, all isolates of *H ducreyi* being found to be sensitive to trimethoprim (Wall RA, unpublished observation). In view of the high proportion of genital ulcers that is due to chancroid, we have recommended that the optimum treatment for this condition in Gambian health centres should be co-trimoxazole and saline washes, as above, combined

Table 2 *Diagnosis of 104 patients*

Diagnosis	No (%) of patients
Chancroid	54 (52)
Syphilis	23 (22)
Lymphogranuloma venereum	7 (7)
Herpes	6 (6)
No diagnosis	28 (27)

Ten (10%) patients had chancroid and syphilis, three (3%) had lymphogranuloma venereum and syphilis, and one had herpes and syphilis.

with a single intramuscular injection of benzathine penicillin 2.4 MIU.

We would like to thank Haddy Sillah for technical help.

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